

Amperometric Detection of Glucose by Polyaniline-Activated Carbon Composite Carbon Paste Electrode

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In this study, a novel carbon paste electrode using the salt form of polyaniline (pani)-activated carbon composite sensitive to glucose, was prepared. Glucose oxidase enzyme was immobilized to modified carbon paste electrode by cross-linking with glutaraldehyde. Determination of glucose was carried out by the oxidation of enzymatically produced H₂O₂ at 0.4 V vs. Ag/AgCl. The effects of pH and temperature were investigated and optimum parameters were found to be 5.0 and 65 °C, respectively. The linear working range of the electrode was 5.0×10⁻⁷ - 1.0×10⁻⁵ M, R²=0.980. The storage stability and operation stability of the enzyme electrode were also studied.

Keywords: Glucose, glucose oxidase, biosensor, polyaniline (Pani), polyaniline activated carbon composite, carbon paste

1. INTRODUCTION

Determination of glucose is essential due to its clinical and industrial importance. Rapid determination of blood sugar for treatment and control of diabetes is significant. Thus; numerous efforts have devoted to develop glucose biosensor with fast and accurate response.

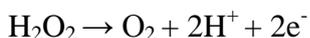
The properties of composite materials depend both the nature of the constituent compounds and their relationships with respect to each other in the final substance. One of the existing composite materials showing electrical conductivity is a carbon paste (CP) [1].

Composite carbon paste electrodes have been extensively used for electroanalytical applications since their introduction by Adams in 1958 [2] due to their intrinsic advantages including low background current, renewable or disposable electrochemical interface and low cost of fabrication

[3]. These electrode materials, has easy fabrication process. They have been involved in the design of amperometric biosensor [4].

For the determination of glucose in many electrochemical glucose biosensors using carbon paste electrodes were prepared [5-7].

Most of the electrochemical glucose biosensors are based on the glucose oxidase (GOx) enzyme, which catalyzes the oxidation of glucose to gluconolactone which was hydrolyzed to gluconic acid and hydrogen peroxide. The quantification of glucose can be achieved via electrochemical detection of the enzymatically released H_2O_2 [8-10].



In this study, a novel carbon paste electrode using the salt form of polyaniline (pani) - activated carbon composite sensitive to glucose, was prepared. Glucose oxidase enzyme was immobilized to modified carbon paste electrode (CPE) by cross-linking with glutaraldehyde. The optimum working conditions of biosensor with respect to the substrate concentration, the pH and temperature were investigated. The storage stability and operation stability of the biosensor were investigated.

2. EXPERIMENTAL SECTION

2.1. Equipment and Reagents

The electrochemical studies were carried out using an Epsilon EC electrochemical analyzer with a three-electrode cell. The working electrode was a carbon paste (diameter of 0.8 cm, length of 3 cm glass tubes) electrode. The auxiliary and reference electrodes were a Pt wire and Ag/AgCl electrode (3 M KCl), respectively. The pH values of the buffer solutions were measured with an Orion Model 720A pH/ion meter. Temperature control was achieved with a Grant W14 thermostat. Glucose oxidase (EC 1.1.3.22, purified from *Aspergillus Niger* and with an activity of 5204.3 unit mL^{-1}) and glucose were purchased from Sigma. Graphite powder and nujol were supplied by Merck and Sigma, respectively. A stock solution of glucose was allowed to mutarotate for 24 h before use. All other chemicals were obtained from Sigma. All the solutions were prepared using double distilled water.

2.2. Preparation of carbon paste electrodes

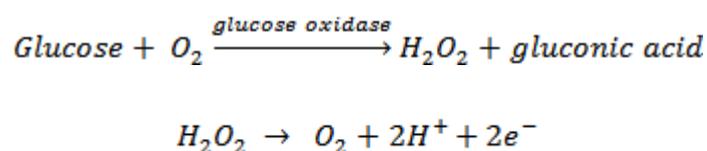
Carbon paste electrode (CPE) was prepared by thoroughly mixing in mortar 100 μL of nujol with 0.15 g of graphite powder. For the preparation of carbon paste electrodes glass tubes (diameter of 0.8 cm, length of 3 cm) were filled with the carbon paste. Height of the paste in the tube was 0.7 cm. Electric contacts were made by platinum wire. The modified carbon paste (MCPE) was prepared with 2 mg polyaniline - activated carbon composite by thoroughly mixing in mortar 100 μL of nujol with 0.15 g of graphite powder. Polyaniline - activated carbon composite was synthesised by Zengin H. and others [11]. The electrode surface was smoothed on a paper to produce a reproducible working surface.

2.3. Preparation of Glucose oxidase/Modified Carbon Paste Electrode (GOx/MCPE)

50 μL glucose oxidase enzyme (5204.3 Unit/mL), 1mg bovine serum albumin, 50 μL 0.1M phosphate buffer at a pH of 5.0 and 30 μL 2.5% glutaraldehyde was dropped upon modified carbon paste electrode. The electrode was dried at room temperature and washed with buffer solution (pH 5.0, 0.1 M phosphate buffer) several times in order to remove the non-immobilized excess enzyme and glutaraldehyde. The electrode was kept in a refrigerator at 4° C in phosphate buffer when it was not in use.

2.4. Electrochemical measurements

The quantification of glucose was achieved via electrochemical detection of the enzymatically released H_2O_2 .



The glucose oxidase/modified carbon paste electrode (GOx/MCPE) was immersed into the phosphate buffer (0.1 M) of pH 5.0. The solution was containing 0.1 M sodium perchlorate as supporting electrolyte. The electrode was brought to equilibrium by keeping at 0.4 V (vs. Ag/AgCl electrode (3 M KCl)). Steady current (i_a) was recorded. Glucose solution was added to the cell from stock solution by using a micropipette, and the system was stirred. The currents (i_b) obtained at 0.4 V were recorded. The current values ($\Delta i = i_b - i_a$) were plotted against the glucose concentrations.

3. RESULT AND DISCUSSION

In this study a novel carbon paste electrode using the salt form of polyaniline (pani)-activated carbon composite sensitive to glucose was prepared. The parameters effecting to the performance of the biosensor and optimum working conditions were investigated.

3.1. Amperometric Responses of CPE and MCPE to Hydrogen Peroxide and Glucose

Amperometric responses of the CP and MCP electrodes to hydrogen peroxide and glucose in the presence of glucose oxidase (50 μL) were determined at different hydrogen peroxide and glucose concentrations. In both cases as shown in figures 1a and 1b, when modified carbon paste was used, current differences were obtained higher than carbon paste. Polyaniline (pani)-activated carbon composite increased the conductivity of carbon paste.

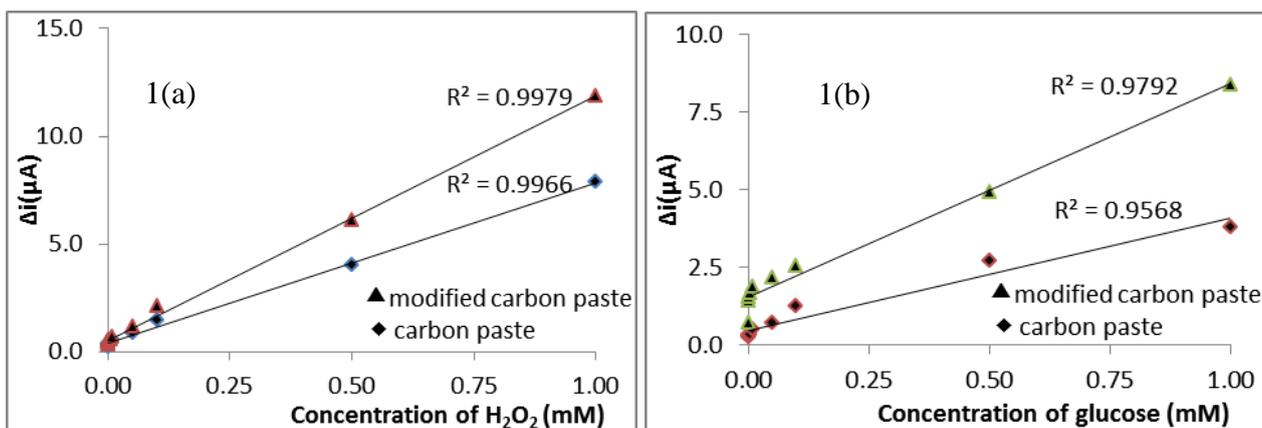


Figure 1. Amperometric responses of carbon paste and modified carbon paste electrodes to hydrogen peroxide (1a) and (1b) glucose in the presence of glucose oxidase (at 25 °C, 0.1 M pH =5.0 phosphate buffer, 0.4 V operating potential)

3.2. The Working Potential

After preparing modified carbon paste electrode (MCPE), the hydrogen peroxide oxidation was carried out at different potentials (0.2, 0.3, 0.4, 0.5, 0.6, 0.7 V) (Fig. 2). When the figure 2 was examined, it was also seen that the variation in current was higher in high potentials than low potentials. It was seen that the linearity of curves in high potentials were higher. The interference effects of substances present in body fluids (e.g., ascorbic acid, uric acid) could be more in high potentials [12]. The variation in current and the linearity of curve in 0.2 V were very low. At 0.3 V, the potential that interference effects could be low, the correlation coefficient was very low. Since the correlation coefficient of the line occurred in 0.4 V was better, 0.4 V were chosen as working potential.

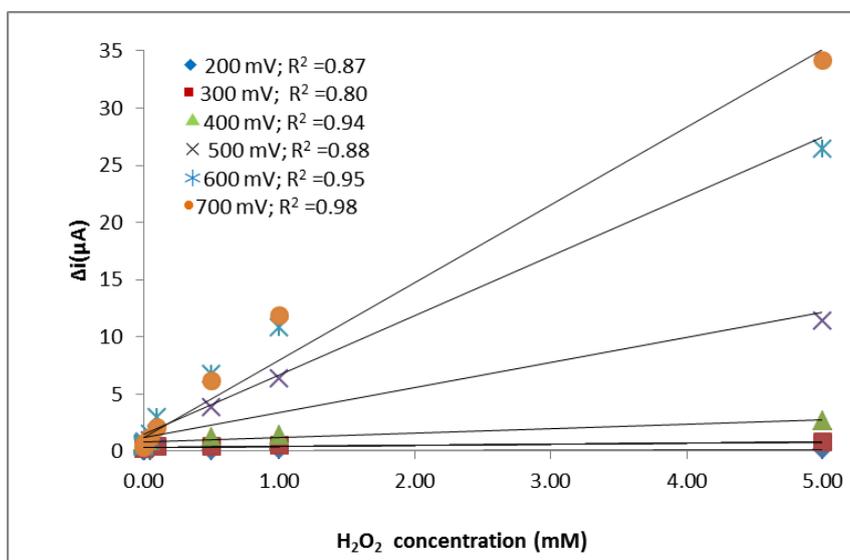


Figure 2. The effect of potential on the response of the modified carbon paste electrode to hydrogen peroxide (at 25 °C, 0.1 M pH =5.0 phosphate buffer, 0.4 V operating potential)

3.3. The effect of Amount of the Polyaniline/Activated Carbon Composite to Selectivity

The selectivity is an important challenge in the development of a biosensor. In order to obtain the best compromise between sensitivity and selectivity, we evaluated the response of the electrode towards ascorbic and uric acids, and acetaminophen [13]. Fig. 3 displays the effect of the amount of composite in the paste on the selectivity of the modified electrode. Additions of 3.0×10^{-4} M uric acid, 1.0×10^{-4} M ascorbic acid and 1.0×10^{-4} M acetaminophen were performed after an initial addition of 5.0×10^{-4} M hydrogen peroxide. The interference was evaluated at 0.4 V using electrodes prepared with 1.0, 2.0, 3.0 mg of polyaniline/activated carbon composite. As it is shown in Fig. 3, the lowest percent ratios of interferences were found when 2 mg composite was used. Therefore, the amount of 2 mg was used in future studies of this composite.

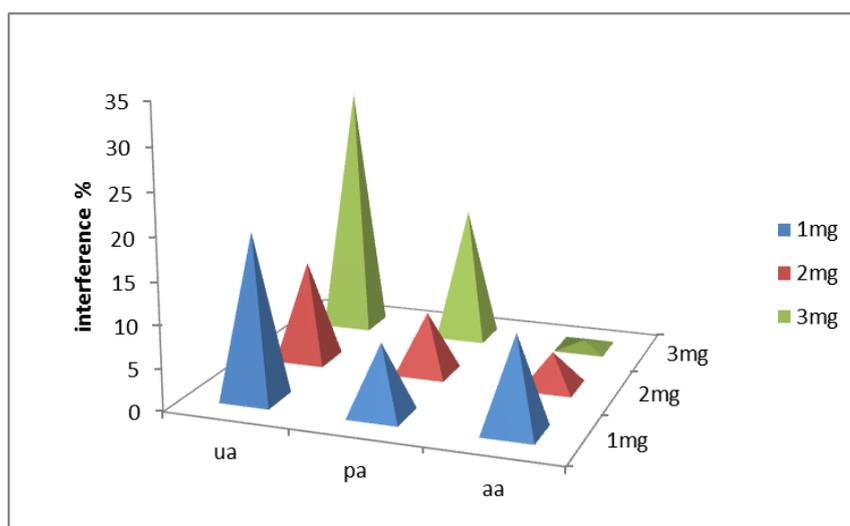


Figure 3. The effect of amount of composite to selectivity (at 25 °C, 0.1 M pH=5.0 phosphate buffer, 0.4 V operating potential)

3.4. Determination of Optimum pH

Since enzyme activity is dependent on the ionization state of the amino acids in the active site, pH plays an important role in maintaining the proper conformation of an enzyme. The effect of pH on the response of the glucose biosensor was determined in 0.1 M phosphate buffer, in the pH range 4.5-10.0. The measurements were performed at a constant glucose concentration of 5.0×10^{-5} M. Figure 4 shows that the maximum response was obtained at pH 5.0. In literature, it can be seen that there are studies that the optimum pH is 5.0 [14, 15]. This could be explained by catalytic activity of leucoemeraldine to reduce oxygen [16]. Polyaniline is known to possess higher conductivity at the solution pH 5.2 as that at the solution pH 7.3. At an operating potential of 440 mV and solution pH 5.0 polyaniline must be partially reduced to leucoemeraldine [17]. For glucose biosensor; pH values different than 5.0 were employed in literature (pH 6.2; 7.5) [18, 19]. This was attributed to the fact that the used polymer and the type of immobilization were different.

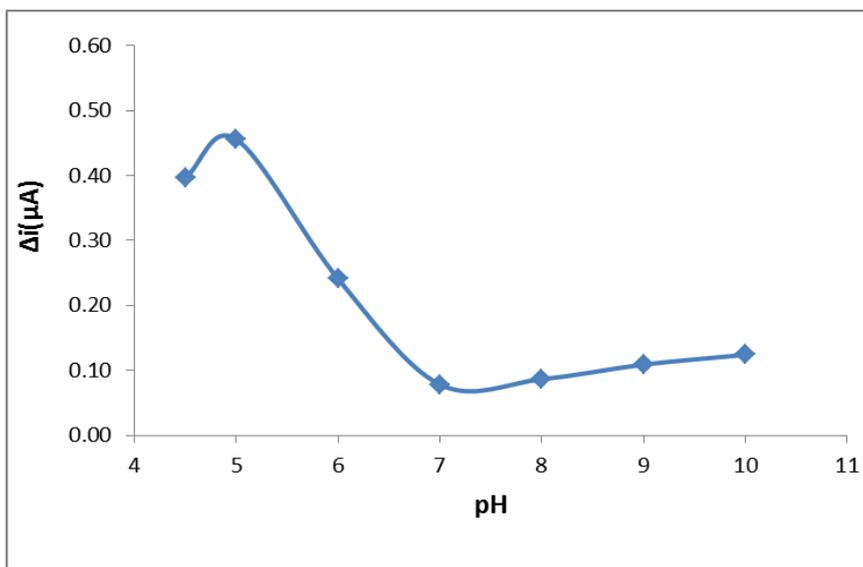


Figure 4. The effect of pH on the response of GOx)/MCPE (at 25 °C, 5.0×10^{-5} M glucose, at 0.4 V operating potential)

3.5. Determination of Optimum Temperature

Enzymes are known to be sensitive to changes in temperature. The relationship between reaction rate of an enzyme and temperature is exponential.

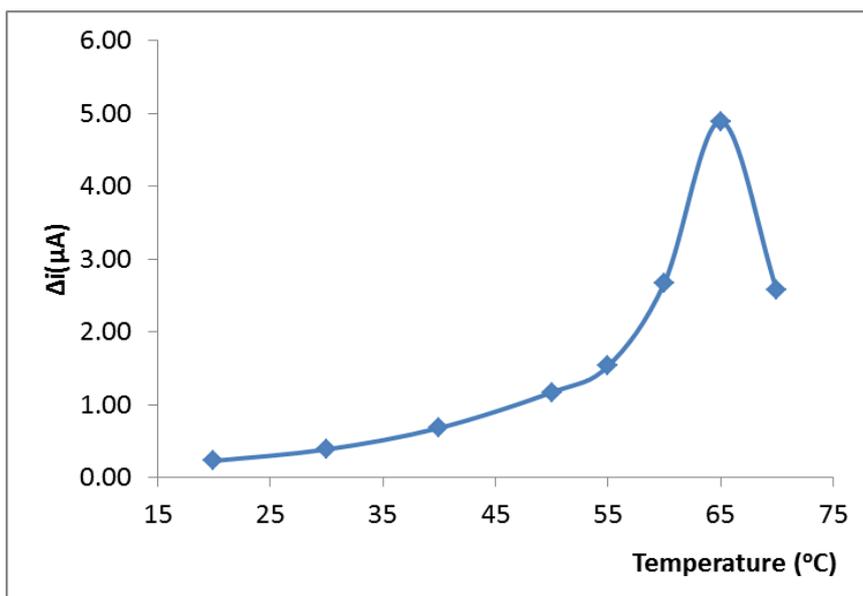


Figure 5. The effect of temperature on the response of GOx)/MCPE (at pH 5.0, 5.0×10^{-5} M glucose at 0.4 V operating potential)

Temperature's influence on the response of glucose enzyme electrode was tested between 20°C and 70°C at pH 5.0 using constant glucose concentration of 5.0×10^{-5} M. As seen from the figure 5, the

current difference increases with temperature up to 65°C and decreases afterwards. The highest electrode response was obtained at 65°C. For glucose biosensor, temperature values were employed in literature (50, 55 °C) [20, 21]. The study was carried out at 25°C due to the difficulties involved in working at 65 °C. The temperature of 25 °C was chosen as working temperature for all further experiments. Glucose oxidase enzyme kept its activity even at high temperatures, when it was immobilized to pani-activated carbon composite.

3.6. Effect of Substrate Concentration on response of GOX/MCP Electrode and Calibration Curve

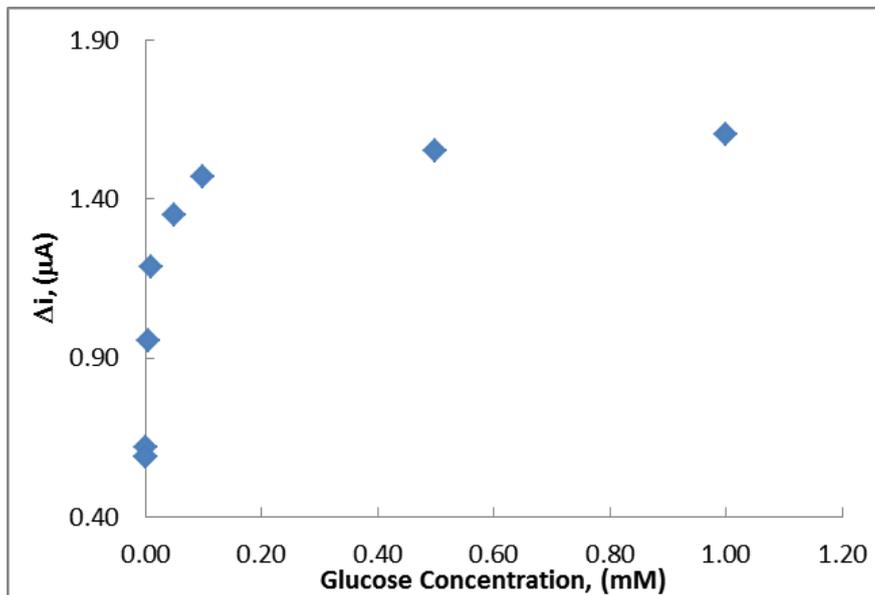


Figure 6. The effect of glucose concentration upon the amperometric response of GOx/MCPE (in pH 5.0 phosphate buffer and at a 0.4 V operating potential, 25 °C)

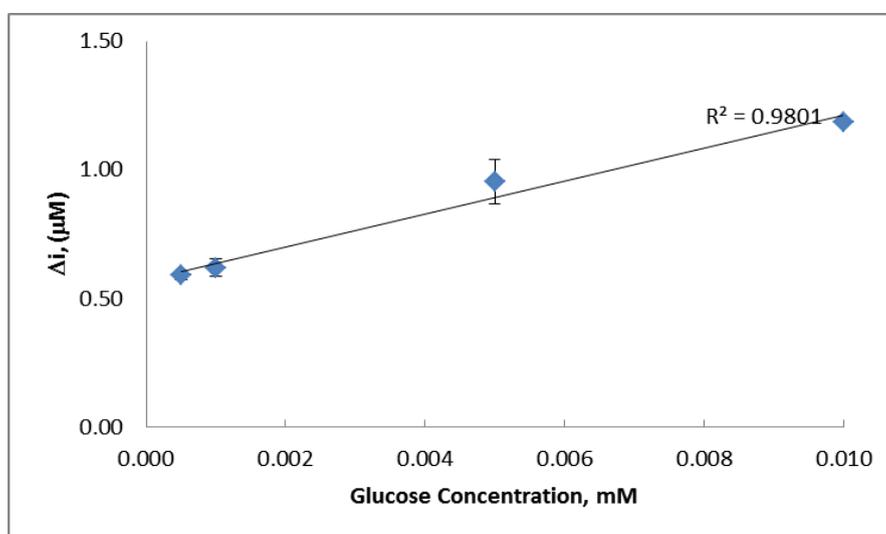


Figure 7. The calibration curve of the glucose biosensor (in pH 5.0 phosphate buffer and at a 0.4 V operating potential, 25 °C)

The effect of the substrate concentration on the reaction rate, catalyzed by immobilized GOD, was studied using varying concentrations ($5.0 \times 10^{-7} - 1.0 \times 10^{-3}$ M) of glucose (Figure 6). The linear working range of the electrode was $5.0 \times 10^{-7} - 1.0 \times 10^{-5}$ M, $R^2 = 0.980$ (Figure 7).

It was shown that the linearity of graphs was highly satisfactory and they could be used for the quantitative determination of glucose. The detection limit of the biosensor was 5.0×10^{-8} M and the response time of the biosensor was 200 s.

Kinetic parameters $I_{\max(\text{app})}$ and $K_{\text{m}(\text{app})}$ for the enzyme biosensor were calculated as 1.22 $\mu\text{A}/\text{min}$ 0.61 mM respectively from Lineweaver–Burk plots (Figure 8). K_{m} values for immobilized glucose oxidase presented in the literature are 20.38, 14.4 mM [21, 22]. This was attributed to the fact that the polymer used and the type of immobilization were different.

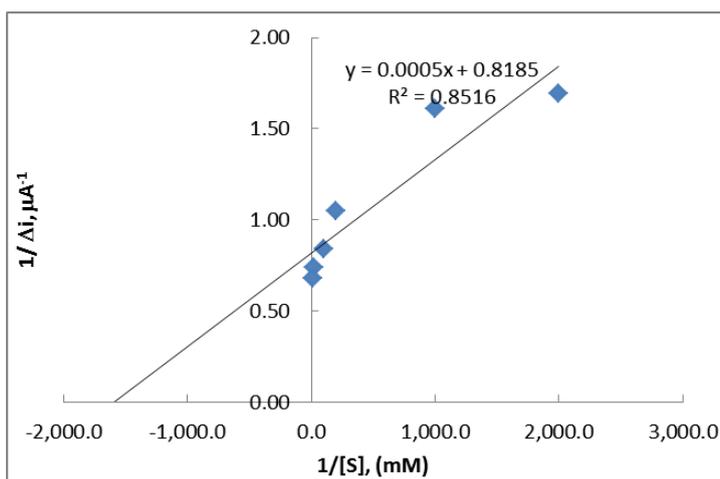


Figure 8. The effect of glucose concentration upon the amperometric response of GOx)/MCPE (Lineweaver-Burk plot, in pH 5.0 phosphate buffer and at a 0.4 V operating potential, 25 °C)

3.7. The Operational Stability of the Enzyme Electrode

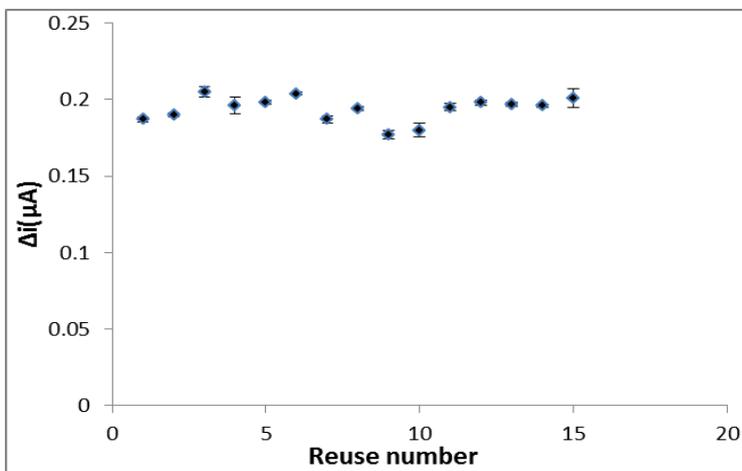


Figure 9. Operational stability of GOx)/MCPE in pH 5.0 phosphate buffer, at a 0.4 V operating potential, 25 °C

The operational stability of GOx/MCPE was studied by performing the activity assay (under optimum conditions) 15 times in the same day (Figure 9). The relative standard deviation obtained after 15 measurements at a constant glucose concentration of 5.0×10^{-5} M was found to be 4.18%.

3.8. The Storage Stabilization of the Enzyme Electrode

The activity assay was applied within 75 days to determine the storage stability of the immobilized enzyme. As shown in Figure 10, during the first 20 days, a decrease was obtained in the response of biosensor. Between the 20th and 75th days, no change was seen in the response of biosensor. An activity loss of 44.8% was observed on the 75th day.

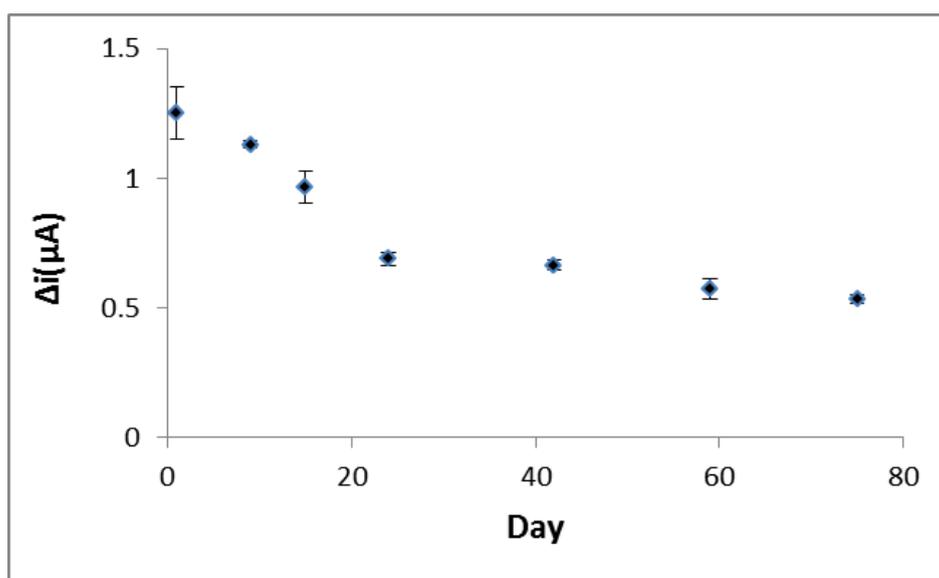


Figure 10. Storage stability of GOx/MCPE (in pH 5.0 phosphate buffer, at 0.4 V operating potential, 25 °C and 5.0×10^{-5} M substrate concentration)

The properties and optimum working conditions for GOx/MCPE is shown in Table 1.

Table 1. The properties and the optimum working conditions of GOx/MCPE

Parameters	Optimum conditions and characteristic
Response time	200 second
Temperature	65°C
pH	5.0
Working range	5.0×10^{-7} - 1.0×10^{-5} M ($R^2=0.980$)
Detection limit	5.0×10^{-8} M
Storage Stabilization	An activity loss of 44.8% was observed on the 75th day.
Operational stability	The relative standard deviation obtained after 15 measurements at a constant glucose concentration of 5.0×10^{-5} M was found to be 4.18%.
$I_{max(app)}$ and $K_{m(app)}$ for Glucose Oxidase enzyme	1.22 $\mu A/min$ and 0.61 mM

4. CONCLUSION

In conclusion, the glucose biosensor prepared in this study:

- Is useable in a large concentration range (5.0×10^{-7} - 1.0×10^{-5} M ($R^2 = 0.980$)).
- Has a very low detection limit (5.0×10^{-8} M).
- Has an acceptable response time for a biosensor (200 s).
- Gives good reproducible results (the relative standard deviation is 4.18% after 15 measurements, the standard deviation obtained).
- Has a long storage stabilization (gives the 55.2 % of the initial amperometric response at the end of the 75th day).
- The $K_{m(\text{app})}$ and $I_{\text{max}(\text{app})}$ values of glucose oxidase enzyme immobilized in Polyaniline/Activated Carbon Composite were 0.61 mM and 1.22 $\mu\text{A}/\text{min}$ respectively.
- It is easy to prepare and highly cost effective.

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